456

VARIATION AND SELECTION WITHIN CLONAL LINES OF *LEMNA MINOR*

A THESIS

PRESENTED TO THE FACULTY OF THE GRADUATE SCHOOL
OF CORNELL UNIVERSITY FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

BY

NEMESIO BLANCO MENDIOLA

STANFORD UNIVERSITY LIBRARIES
WITHDRAWN

Reprinted from GENETICS 4: 151-182, March, 1919

3781
C81M

VARIATION AND SELECTION WITHIN CLONAL LINES OF *LEMNA MINOR*

A THESIS

PRESENTED TO THE FACULTY OF THE GRADUATE SCHOOL
OF CORNELL UNIVERSITY FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

BY

NEMESIO BLANCO MENDIOLA

Reprinted from GENETICS 4: 151-182, March, 1919

VARIATION AND SELECTION WITHIN CLONAL LINES OF *LEMNA MINOR*[1]

NEMESIO BLANCO MENDIOLA
University of the Philippines, Manila, Philippine Islands

[Received July 24, 1918]

TABLE OF CONTENTS

PAGE
INTRODUCTION 151
Material used 151
Variation within a wild population 152
Shape of frond 152
Speed of budding 154
Variation in the habit of root growth 156
Variation in size of fronds 158
Variation and selection in clonal lines 159
Variation and selection in shape of frond 159
Effect of culture media 160
Inheritance of shape within a clone 161
Unusual non-heritable variations in frond shape 164
Clonal variation and selection in speed of propagation 164
Clonal variation in size of frond 169
Effect of culture media on clonal size variation 172
Inheritance of acquired size 173
Inheritance of decreased size 173
Inheritance of increased size 174
Clonal selection for size of frond 174
DISCUSSION AND CONCLUSIONS 180
SUMMARY 182
LITERATURE CITED 182

INTRODUCTION

The experiments herein reported were conducted for the purpose of adding to our knowledge concerning clonal variation and the effect of selection on such variation, in relation to the pure-line concept. It is unnecessary here to include a review of published data. It is, of course, generally recognized that the greater bulk of experimental evidence supports the pure-line theory as elaborated by JOHANNSEN.

MATERIAL USED

The plants used in these experiments are commonly called duckweed

[1] Paper No. 73, Department of plant breeding, CORNELL UNIVERSITY, Ithaca, N. Y.

and belong to the genus Lemna. This, according to GRAY (1908), is a genus widely distributed over Europe, Northern Asia and North America, but rare in the Tropics. The duckweeds are small, floating plants without distinct stems or real leaves, and may or may not have roots. They rarely produce flowers, the usual mode of propagation being through budding. The present paper is concerned only with one species, *Lemna minor* Linn.

It is necessary to give a more than passing statement regarding the mode of budding. The main structure of the plant is usually called a frond. Some botanists regard it either as a stem, or leaf, or both fused together. The term "frond" is used throughout this paper. According to BLODGETT (1915) the frond consists of three parts: (a) a terminal leaf, (b) a bud rudiment inclosed by a flattened bud scale and (c) an apical region from which new fronds are developed. Vertical pressure during the early stages of growth causes the splitting of the bud rudiment into two buds which do not develop at the same time. These outgrowths come out as a horizontal series in an overlapping form through the lack of space for vertical succession. The development of the basal region into a stalk or stipe causes the thrusting forward of each new whole structure. In *L. minor* this basal region is attached marginally to the main portion of the frond; in other species, as in *L. polyrrhiza,* it is inserted upon the vertical surface some distance from the edge. Figure 1 shows a parent frond with its offspring still attached to it. The members of the family are numbered consecutively in the order of the time of their appearance.

VARIATION WITHIN A WILD POPULATION

Before studying clonal variations a study within a wild population was made concerning shape and size of fronds, speed of propagation and root habits.

Shape of frond

Figure 2 shows fronds of various shapes taken from a population which was collected on December 10, 1916, from a stagnant creek at the Ithaca fair-grounds. The sketches were made by examining the specimens under Zeiss binoculars and tracing the outlines of the image as thrown over the paper with the aid of a Zeiss camera lucida. In all cases mature fronds, such as had already turned yellow but which were still attached to their offspring were studied, thus eliminating, as far as possible, the effect of different ages. From the figure just referred to it

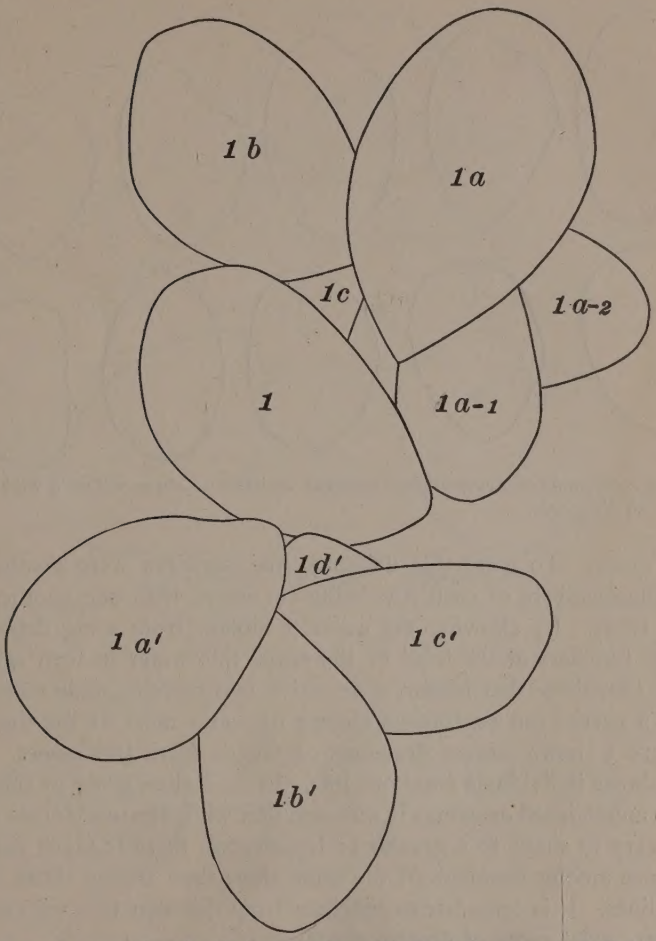

FIGURE 1.—A parent frond with its offspring still attached to it. × 16 diameters.

may be seen that there exist diverse forms of fronds in a wild population. To determine whether or not differences in shape are inherited, that is, to ascertain if different forms represent distinct strains, several fronds were isolated from the wild stock. Each frond was allowed to propagate in a tumbler containing tap water and kept in a greenhouse section in which the temperature was generally 15° C at night and 25° C by day. Preliminary cultural experiments had shown that the plants die after a time if frequent change of water in the culture tumblers

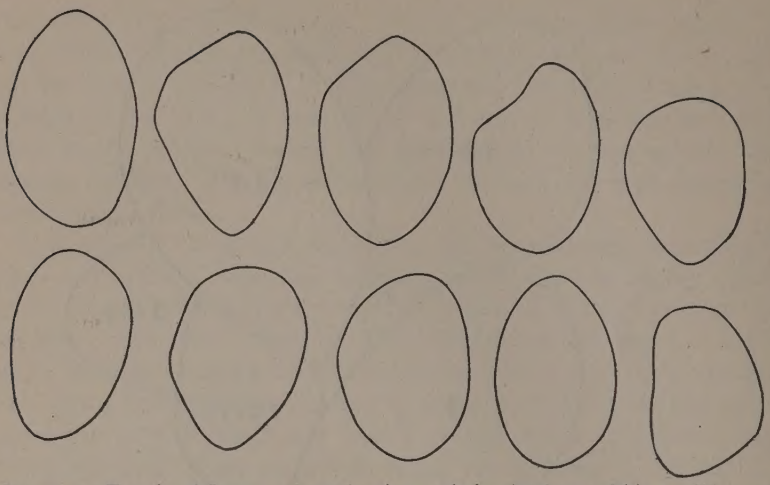

FIGURE 2.—Fronds of *Lemna minor* showing variation in shape within a wild population. × 16 diameters.

is not made. To meet this difficulty, the tumblers were arranged in rows, the members of each row being connected with one another with siphon tubes. By allowing the water to siphon from a big deposit jar into the tumblers at the head of the rows, this water in turn being siphoned into those that follow, a provision was thereby made which permitted a partial but continuous change of water most of the time.

Figure 3 shows camera drawings of fronds from two clones. Each figure shows individuals from one line. From a close study of these and similar unpublished drawings it was seen that while the individuals within a line vary in shape to a greater or less degree, there is much more resemblance among members of the same clone than among those of different lines. It is only fair to conclude from this that in a wild population there exist races of diverse shape.

Speed of budding

The term "speed" does not imply "rate." There is no use of studying variation in rate of budding in *L. minor* since different fronds have the same rate of budding. Each frond produces invariably two buds and no case has yet been reported where more or less than this number has been produced. However, different fronds may require different lengths of time to produce their offspring buds. Speed of budding may be measured either by noting the number of days it takes for a given number

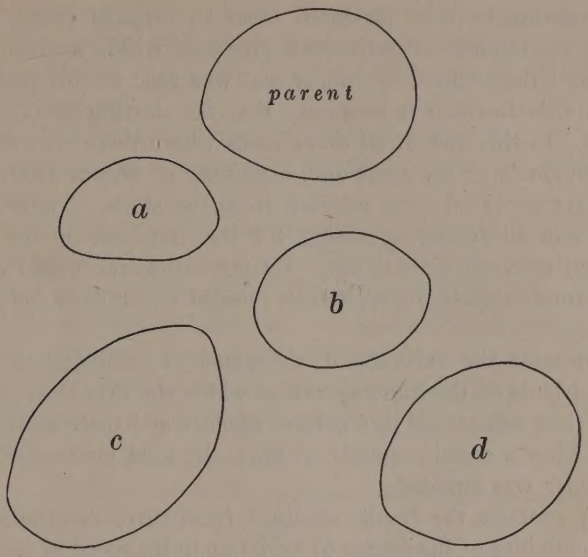

FIGURE 3.—Fronds from clones 18 and 26. × 16 diameters.

of new individuals to be produced from an original frond, or by determining the number of individuals produced within a given length of time. The latter method is simpler and was used in this study.

In this experiment it is necessary that the starting fronds be of the same age. In this and in all other cases where there was necessity of using individuals of the same age, a number of fronds from which no bud had yet appeared were selected from the stock. These were then observed and all fronds appearing for the first time on the same day were taken to be of similar age. By increasing the initial number of starting fronds almost any reasonable number of similar-aged buds could be obtained.

To determine the variation in the speed of propagation, each of a number of buds of the same age from which the first buds appeared at the same time was placed in a culture tumbler and there allowed to propagate. After a certain number of days, the total number of fronds in each tumbler was counted.

Table 1 contains the results obtained from three determinations and gives a rough idea of the degree of variation in the speed of reproduction.

TABLE 1
Variation in speed of reproduction.

Class values	Frequency		
	Dec. 30-Jan. 9	Feb. 20-Mar. 2	Feb. 25-Mar. 7
3	0	0	0
4	5	2	3
5	8	8	3
6	14	8	15
7	22	26	30
8	7	10	4
9	3	5	2
10	1	1	3
Mean	6.517 ± .115	6.883 ± .110	6.783 ± .107
σ	1.323 ± .081	1.266 ± .078	1.266 ± .075
C. V.	20.30 ± 1.30	18.39 ± 1.16	18.07 ± 1.14

The variations shown in the preceding table do not appear to be multimodal and do not indicate that they represent different speed strains.

Variation in the habit of root growth

It is commonly observed that there is a tendency for plants of *L. minor* to produce curly or twisted roots. The manner of this curling or twist-

ing is by no means uniform. While in general the curling is only immediately below the tip, other plants have longer portions of their roots in a twisted condition. In a few cases, the twisting may even come to the middle of the root.

The value of this habit of the plant as a character for the study of variation depends upon whether it is hereditary or is merely the effect of environment.

Unfortunately, variation in this character cannot be measured with any degree of accuracy and does not lend itself readily to genetical study. What is worse, it makes the study of the variation in size, such as in length of the roots almost impossible. An attempt was made to grow a number of the duckweeds on 2 percent agar-containing nutrient solution, hoping to get straight roots which would lend themselves to measurements, but this attempt failed, the roots refusing to grow or sink into the agar media.

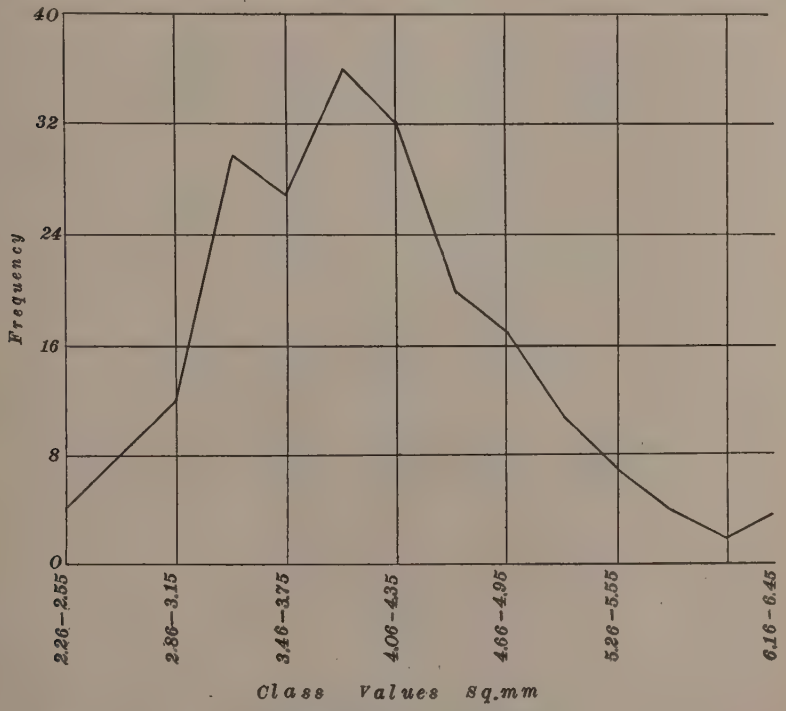

FIGURE 4.—Curve showing variation in size in a population of *L. minor*.

Variation in size of fronds

The size of a frond was determined by measuring its camera-magnified area with the aid of a small planimeter and then computing the true area by dividing the magnified area by 256, the number of times the object was magnified. Two hundred mature-population fronds which were of the same age and which matured at the same time were so measured. Table 2 gives the results of the measurement and figure 4 shows the frequency curve. The curve shows a tendency to three modes, one of these occurring at 3.16-3.45 mm², another at 3.76-4.05 mm², and a third at 6.16-6.45 mm². It might be concluded from this that in a population of *L. minor* there is a probability of the existence of diverse size strains. Such diverse strains need not be found in all localities since the extreme rareness with which this plant has a chance to cross-breed and the rapidity with which it reproduces by budding, both tend, with the help of natural selection, to reduce the inhabitants of a locality to that of a clonal line. None of the clonal lines studied showed a bimodal condition.

Following the determination of the frequency distribution shown in table 2 it would have been only logical to ascertain whether the size modes persist, that is, whether or not the size races found are permanent. An attempt was made to do this. It was planned to isolate several lines representing widely different sizes and then to determine at different intervals of time the average of each line. This attempt, however, was unsuccessful. It was found that *L. minor* cannot be grown successfully in tap water for several months in spite of frequent change of this medium. After a month or so, the fronds usually begin to decrease in size and by the time when enough individuals are needed to give a fair sample, the lines usually have run out. As will be learned later in this paper, continuous culture was maintained by the use of a mineral nutrient solution. It was deemed unwise, however, to use this culture in such an experiment as the determination of the persistence of size differences, since, as will soon be seen, mineral solution had a decided effect in increasing the size of the fronds and no form of culture check could be devised with which this effect could be controlled.

TABLE 2

Distribution of variation in the size of 200 fronds from a wild population of Lemna minor.

	Class values in square millimeters													Mean	σ	C. V.
	2.26-2.55	2.56-2.85	2.86-3.15	3.16-3.45	3.46-3.75	3.76-4.05	4.06-4.35	4.36-4.65	4.66-4.95	4.96-5.25	5.26-5.55	5.56-5.85	5.86-6.15	6.16-6.45			
Frequency	3	7	11	29	26	35	31	19	16	10	6	3	1	3	4.019±.037	.767±.026	16.60±.570

VARIATION AND SELECTION IN CLONAL LINES

Variation and selection in shape of frond

It has been seen already, in the discussion of the permanence of shape strains, that different clones with distinctly different-shaped fronds tend to reproduce their respective characteristic shapes. A certain amount of variation in shape within the clones was also pointed out. Further studies along this line were carried out. The plants, as previously, were grown in tumblers, but in mineral nutrient solution instead of tap water. The use of this solution made the continuous change of culture media unnecessary. The nutrient solution was prepared according to the following modified formula of PFEFFER:

Constituents	Grams per liter
$Ca(NO_3)_2$	0.4
NaCl	0.1
$MgSO_4$	0.1
KH_2PO_4	0.1
$Fe_3(PO_4)_2$	0.1
KNO_3	0.1

To study the variation in shape, one hundred mature fronds grown

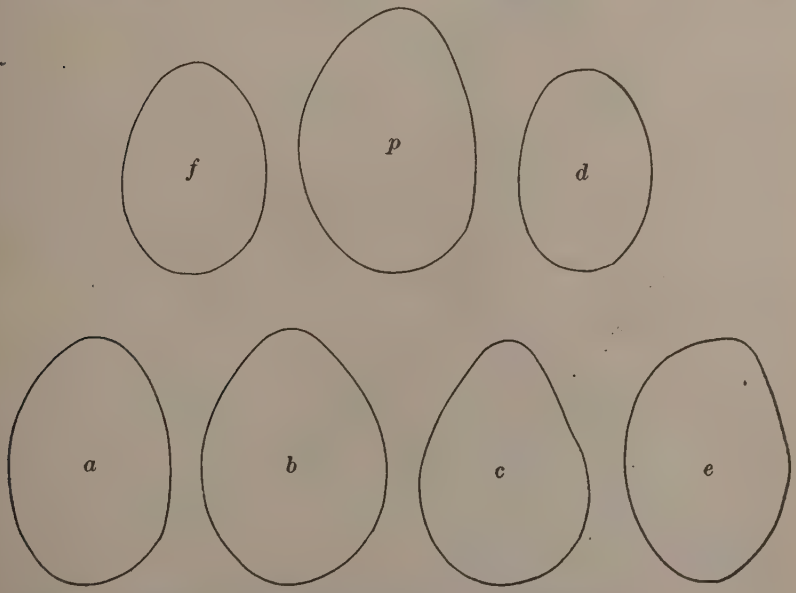

FIGURE 5.—Variation in frond shape in clone 35. × 16 diameters.

from buds of the same age were drawn for each clone. The resulting drawings were classified according to shapes. Figure 5 will give some idea of the dominant shape and the shapes of the varying individuals in clone 35. This dominant shape is represented by the letter p, while the varying shapes are represented by letters a, b, etc.

The frequency of the different shape types in four clones studied is given in table 3.

TABLE 3
Frequency of shape variants.

Clone	Shape types							Total number of individuals
	p	a	b	c	d	e	f	
35	51	3	22	17	1	2	4	100
	Shape types							
	p	g	h	i	j	k	l	
38	47	21	13	11	5	2	1	100
	Shape types							
	p	m	n	o	q	r		
76	48	7	10	15	18	2		100
	Shape types							
	p	s	t	u	v	w		
81	46	14	13	13	1	13		100

From table 3 it may be seen that in clonal lines there exist different shapes of fronds, with some one type predominating.

Before taking up the subject of inheritance in shape in clonal lines, it is well to discuss the results of the study of several of the factors affecting variation.

Effect of culture media

Before this part of the experiment was undertaken, it had been observed that fronds growing in tap water had a decidedly different appearance from those growing in nutrient solution. This was partly due to a difference in size; those growing in nutrient medium were very much larger than those in tap water. Suspecting that there may be also a difference in general shape in the two cultures, it was decided to carry out experiments along this line. Clones 38, 39, 41 and 79 were used. Parallel cultures were set up for each clone. Initial buds of those grown

in tap water came from stock growing in tumblers containing water and garden soil, while buds of those grown in nutrient solution came from stocks already growing in nutrient medium. The four series were not grown at the same time as were the paired cultures from each clone. From each culture one hundred mature fronds were harvested, drawn, and classified according to shape. Table 4 gives the frequency of the different types observed.

TABLE 4

Frequency of shape types of plants grown in tap water and in nutrient solution.

Clone 38			Clone 39			Clone 41			Clone 79		
Types	Tap water	Nutrient solution	Types	Tap water	Nutrient solution	Types	Tap water	Nutrient solution	Types	Tap water	Nutrient solution
a	18	28	i	24	36	o	0	8	u	27	41
b	20	3	j	16	18	p	49	12	v	15	17
c	7	12	k	0	17	q	28	54	w	10	10
d	2	1	l	12	4	r	16	11	x	0	1
e	33	7	m	8	13	s	7	9	y	48	20
f	0	1	n	40	12	t	0	6	z	0	11
g	0	3									
h	20	45									
Total number	100	100		100	100		100	100		100	100

Table 4 shows two important points: (1) in every case there was found greater variation in nutrient-grown plants than in those grown in tap water, and (2) the predominant shape in each clone is different for the two culture media. In clone 38, for example, shape e was predominant among the tap-water-grown plants while among those grown in nutrient solution shape h was the predominating type.

Inheritance of shape within a clone

It has already been pointed out (see table 3) that a study of one hundred mature fronds of clone 81 revealed six shape types, s to w, with type p predominating. To determine to what extent these different shape types are hereditary, a family was bred from each type in nutrient solution and one hundred mature fronds from each were drawn and studied as to variation in shape. Table 5 contains the results of this study.

We see from table 5 that the parental type seems to have had no effect on the type distribution (excepting the type representing the clone. An interesting fact brought out by the above data is that while the diverse shapes which do not represent that of the clone were not hereditary, they

TABLE 5

Frequency of types in different families of clone 81.

Parent types	Types of progeny and their distribution										
	s	t	p	u	v	w	g	h	i	j	k
s	1	7	43	13	1	17	0	6	2	6	4
t	0	6	36	17	1	9	7	12	1	5	6
p	3	8	51	9	1	9	5	5	1	6	2
u	1	6	47	3	0	30	0	4	1	8	0
v	1	6	47	9	2	19	3	8	1	3	1
w	2	8	41	7	1	25	2	10	1	3	0

appeared in approximately the same relative proportion to one another irrespective of their parental shapes.

In order that this point may be seen more clearly, the data in table 5 were made into curves shown in figure 6.

Another attempt to change the dominant shape type of clone 81 was made by continuous selection of shapes u and w. The experiment was carried through three periods, each period comprising many generations. There were three cultures during each period, one for u selection, one for w and another for p. The latter served as control. One hundred mature fronds were examined from each harvest. Table 6 contains the results.

TABLE 6

Results of continuous selection for types u and w in clone 81.

Parent shapes	Shapes of progeny and distribution										
	s	t	p	u	v	w	g	h	i	j	k
	First period										
u	1	5	49	10	2	25	1	2	2	3	0
p	3	5	48	11	2	21	1	2	2	5	0
w	7	3	46	4	2	20	2	7	2	7	0
	Second period										
u	6	12	38	5	0	27	0	10	2	0	0
p	3	13	35	5	1	31	3	8	1	0	0
w	10	17	26	4	2	22	6	7	6	0	0
	Third period										
u	17	0	50	12	5	8	3	2	3	0	0
p	13	7	37	18	3	22	0	0	0	0	0
w	7	5	29	16	7	17	8	7	4	0	0

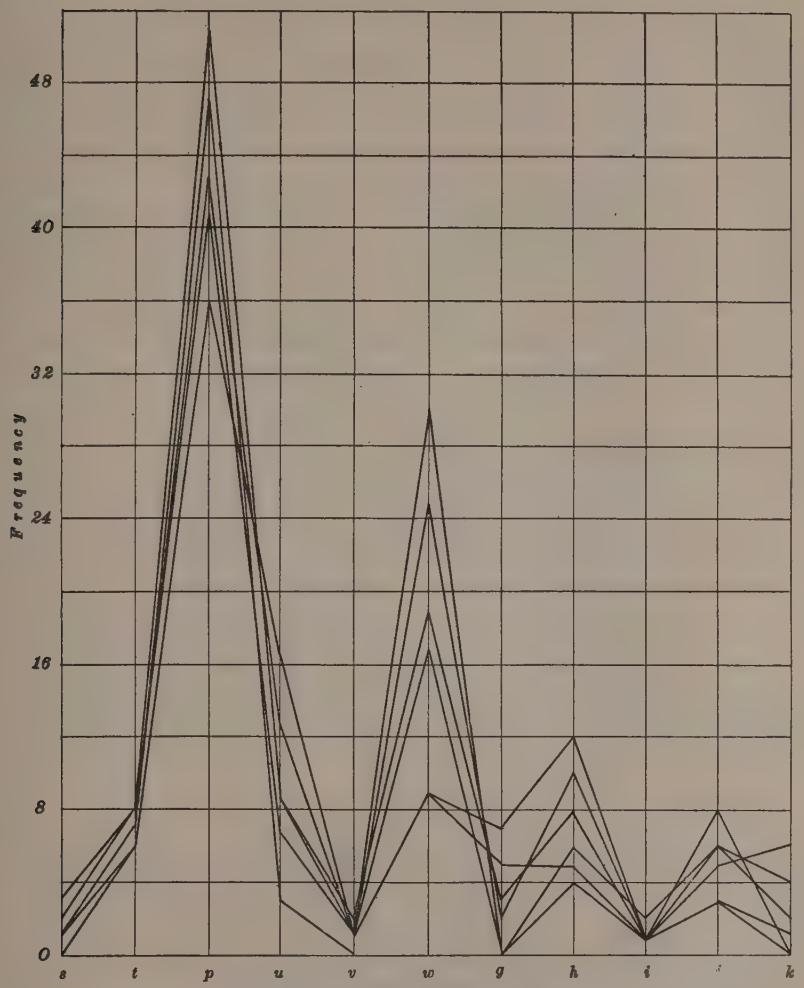

FIGURE 6.—Frequency curves of different shape types in different families of clone 81.

It would appear from the results shown in tables 5 and 6 that the different non-dominant shape types in clone 81 are merely somatic variations, probably physiological, and are not inheritable, and that selection for these different shapes has made no progress. As early as 1894, GUPPY (1894) reported that long exposure to different habits of life, as growing in mud, had not produced any permanent change in the external appearance of duckweeds.

Unusual non-heritable variations in frond shape

During the entire period of investigation, a watch was continually kept for mutations. Three fronds of unusual shapes appeared in nutrient cultures, two in clone 41 and one in clone 42. When they were found, they were still attached to their parent fronds. Each of these unusual-shaped fronds together with each parent was placed in a separate culture tumbler and allowed to propagate to determine if they were mutations. When matured individuals in each tumbler numbered fifty or more, the cultures were discontinued and the mature fronds examined. It was found that none of these aberrant shapes was hereditary.

Selection in opposite directions was made in each of the four clones mentioned above. Each selection was carried through five periods. Plus selection was made by continuous selection of individuals falling in classes 9 and 10, and minus selection, of those in classes 5 and 4. A check culture of unselected individuals was also grown. The three cultures in each clone—plus, minus and check,—were always grown at the same time. The plants were grown in the nutrient medium and good care was taken to render cultural and other controllable conditions as much alike as possible for each series. Tables 8-11, inclusive, show the results of this selection, and table 12 contains the differences between the means of the check cultures and those of the plus and minus selections. If the selection be effective, there should be an increasing difference between the means of the check and selection series from the first to the last period of the experiment.

Clonal variation and selection in speed of propagation

In this study, clones 38, 39, 78 and 81 were used. The unit of time taken was 11 days. Sixty individuals were studied in each culture. Initial studies of variation in speed of propagation of these different lines gave results which are shown in table 7. A "class value" in this case represents the total number of individuals obtained by allowing an original bud and its offspring to propagate during 11 days.

TABLE 7
Clonal variation in speed of propagation.

Clone	Period	Class values									Mean	σ	C.V.
		3	4	5	6	7	8	9	10	11			
38	May 18-June 18	0	3	6	8	23	11	8	1	0	7.017±.118	1.360±.084	19.38±1.24
39	May 18-June 18	1	2	2	6	24	11	8	5	1	7.433±.133	1.532±.094	20.61±1.32
78	June 11-June 21	0	3	7	11	25	10	3	1	0	6.750±.109	1.247±.077	18.47±1.17
81	June 19-June 29	0	2	3	12	27	10	4	2	0	7.000±.104	1.197±.074	17.10±1.08

From table 7 it is to be seen that clones 38 and 81 seem to have the same speed of budding. The other two clones, however, appear to possess distinctly different means, whose difference is .683±.172, so that it may be considered as highly probable that in a population of *Lemna minor,* there exist also different strains in regard to speed of asexual propagation.

TABLE 8

Selection in speed of propagation in clone 38.

Selection	Frequency of class values									Mean	σ	C.V.
	3	4	5	6	7	8	9	10	11			
First period												
Plus (+)		2	7	15	26	8	2			6.617±.093	1.066±.066	16.11±1.02
Check		1	7	14	27	9	2			6.700±.082	1.021±.063	15.24±0.96
Minus (—)		1	10	12	25	10	2			6.650±.095	1.093±.067	16.44±1.04
Second period												
Plus (+)			8	15	28	6	3			6.683±.086	0.991±.061	18.83±0.93
Check			7	15	27	8	3			6.750±.087	0.994±.061	14.72±0.92
Minus (—)		3	8	17	23	6	3			6.500±.100	1.147±.071	17.65±1.12
Third period												
Plus (+)		3	8	13	26	8	2			6.567±.098	1.131±.070	17.22±1.09
Check		1	11	15	26	5	2			6.483±.091	1.041±.064	16.06±1.01
Minus (—)		3	6	14	25	10	2			6.650±.098	1.123±.069	16.89±1.07
Fourth period												
Plus (+)		3	4	10	25	12	6			6.950±.106	1.217±.075	17.52±1.11
Check		3	8	10	25	11	2	1		6.717±.108	1.240±.076	18.46±1.17
Minus (—)		2	4	11	24	11	5	3		7.083±.114	1.308±.081	18.47±1.17
Fifth period												
Plus (+)		2	8	12	26	9	2	1		6.700±.102	1.173±.072	17.51±1.11
Check		1	10	14	25	7	3			6.600±.096	1.098±.068	16.64±1.05
Minus (—)		3	9	13	24	9	2			6.550±.101	1.161±.071	17.72±1.12

TABLE 9

Results of continuous selection in speed of propagation in clone 39.

Selection	Frequency of class values										Mean	σ	C. V.
	3	4	5	6	7	8	9	10	11	12			
First period													
Plus (+)		3	3	7	30	8	6	3			7.117±.115	1.318±.081	18.52±1.18
Check		2	4	4	30	8	8	3	1		7.317±.121	1.384±.085	18.91±1.20
Minus (—)		2	6	6	29	10	4	2	1		7.067±.117	1.340±.083	18.96±1.21
Second period													
Plus (+)	1	4	6	28	11	9	1				6.250±.103	1.178±.073	18.85±1.20
Check	2	2	5	29	9	7	5	1			6.450±.122	1.396±.086	21.64±1.39
Minus (—)	3	3	4	27	12	10	1				6.267±.112	1.289±.079	20.57±1.32
Third period													
Plus (+)		3	2	8	26	10	6	4	1		7.283±.125	1.439±.089	19.76±1.26
Check	1	2	3	9	25	9	6	5			7.183±.129	1.478±.091	20.58±1.32
Minus (—)		3	4	7	26	8	7	4	1		7.233±.131	1.499±.092	20.72±1.33
Fourth period													
Plus (+)		1	2	8	28	9	6	5	1		7.417±.117	1.345±.083	18.13±1.15
Check	2	0	3	6	29	10	7	3			7.217±.119	1.367±.084	18.94±1.21
Minus (—)	1	4	4	5	31	7	5	2	1		6.967±.130	1.494±.092	21.44±1.38
Fifth period													
Plus (+)			1	8	21	14	6	7	3		7.817±.124	1.420±.087	18.16±1.15
Check	1	0	3	3	25	10	4	7	5	2	7.933±.158	1.815±.112	22.87±1.48
Minus (—)		2	3	4	25	10	8	5	3		7.617±.136	1.561±.096	20.49±1.31

TABLE 10

Results of continuous selection in speed of propagation in clone 78.

Selection	Frequency of class values									Mean	σ	C.V.
	3	4	5	6	7	8	9	10	11			
First period												
Plus (+)		1	7	21	22	7	2			6.550±.088	1.007±.062	15.37±0.97
Check		1	10	21	25	3	0			6.317±.075	0.866±.053	13.71±0.86
Minus (—)		2	10	16	27	3	2			6.417±.090	1.038±.064	16.17±1.02
Second period												
Plus (+)		1	7	11	25	11	4	1		6.900±.103	1.179±.073	17.09±1.08
Check	1	3	5	6	29	9	5	2		6.933±.121	1.389±.086	20.03±1.28
Minus (—)		2	6	10	30	7	4	1		6.833±.102	1.171±.072	17.13±1.08
Third period												
Plus (+)			5	17	31	5	2			6.700±.075	0.862±.053	12.86±0.80
Check	1	2	6	14	27	8	2			6.600±.100	1.143±.070	17.32±1.10
Minus (—)		5	7	13	24	8	3			6.533±.108	1.245±.077	19.06±1.21
Fourth period												
Plus (+)		1	5	10	29	11	3	1		6.950±.095	1.087±.067	15.64±0.99
Check		1	3	7	31	12	4	2		7.167±.102	1.171±.072	16.33±1.03
Minus (—)		2	4	10	27	12	4	1		6.983±.101	1.162±.072	16.64±1.05
Fifth period												
Plus (+)		2	5	9	26	13	3	2		7.000±.107	1.225±.075	17.50±1.11
Check	1	1	5	8	28	12	4	1		6.967±.107	1.224±.075	17.57±1.11
Minus (—)		1	8	8	30	10	2	1		6.833±.097	1.113±.069	16.29±1.03

TABLE 11

Results of continuous selection in speed of propagation in clone 81.

Selection	Frequency of class values									Mean	σ	C.V.
	3	4	5	6	7	8	9	10	11			
First period												
Plus (+)	5	7	10	13	22	2	1			5.833±.123	1.416±.087	24.27±1.58
Check	6	8	10	13	20	2	1			5.717±.127	1.462±.090	25.57±1.67
Minus (—)	8	10	12	23	5	2				5.217±.113	1.292±.080	24.76±1.61
Second period												
Plus (+)	2	3	5	12	30	4	3	1		6.567±.115	1.321±.081	20.12±1.29
Check		1	5	6	33	8	3	3	1	7.133±.111	1.271±.078	17.82±1.13
Minus (—)	1	3	4	7	34	6	3	2		6.833±.113	1.293±.080	18.92±1.20
Third period												
Plus (+)		2	1	10	25	13	5	4		7.283±.110	1.266±.078	17.38±1.10
Check		1	2	11	28	10	6	1	1	7.183±.104	1.190±.073	16.57±1.05
Minus (—)		1	4	7	31	9	5	2	1	7.183±.108	1.245±.077	17.33±1.10
Fourth period												
Plus (+)		1	4	17	26	6	5	1		6.850±.098	1.123±.069	16.39±1.03
Check		1	5	20	24	5	4	1		6.717±.097	1.112±.068	16.55±1.05
Minus (—)		1	2	12	28	10	5	2		7.117±.098	1.127±.069	15.83±1.00
Fifth period												
Plus (+)		1	4	6	32	9	5	2	1	7.200±.108	1.236±.076	17.16±1.09
Check		1	3	10	29	8	6	2	1	7.183±.110	1.258±.077	17.51±1.11
Minus (—)		1	5	5	30	8	7	3	1	7.283±.117	1.343±.083	18.44±1.17

TABLE 12

Differences between the means of the check and those of the selections.

Period	Difference between means of check and plus selections	Difference between means of check and minus selections
	Clone 38	
First	—0.083 ± .124	0.050 ± .125
Second	—0.067 ± .122	0.250 ± .133
Third	0.084 ± .134	—0.167 ± .134
Fourth	0.233 ± .151	—0.366 ± .157
Fifth	0.100 ± .140	0.050 ± .139
	Clone 39	
First	—0.200 ± .167	0.250 ± .168
Second	—0.200 ± .160	0.183 ± .166
Third	0.100 ± .179	—0.050 ± .184
Fourth	0.200 ± .167	0.250 ± .176
Fifth	—0.116 ± .201	0.316 ± .209
	Clone 78	
First	0.233 ± .115	—0.100 ± .117
Second	—0.033 ± .159	0.100 ± .158
Third	0.100 ± .125	0.067 ± .147
Fourth	—0.217 ± .139	0.184 ± .144
Fifth	0.033 ± .151	0.134 ± .144
	Clone 81	
First	0.116 ± .177	0.500 ± .170
Second	—0.566 ± .160	0.300 ± .158
Third	0.100 ± .151	0.000 ± .150
Fourth	0.133 ± .138	—0.400 ± .138
Fifth	0.017 ± .154	—0.100 ± .161

From the data in table 12 it may be concluded that there was no progress obtained in either the plus or minus selection for speed of budding.

Clonal variation in size of frond

As a preliminary selection study the variation in size of fronds in four clones was studied. Selection was later performed in these same four lines. The plants were grown in nutrient solution contained in

Table 13

Variation in size in square millimeters in clonal lines of Lemna minor.

Clone	Class values and frequencies																				Mean	σ	C. V.
	1.76-2.25	2.26-2.75	2.76-3.25	3.26-3.75	3.76-4.25	4.26-4.75	4.76-5.25	5.26-5.75	5.76-6.25	6.26-6.75	6.76-7.25	7.26-7.75	7.76-8.25	8.26-8.75	8.76-9.25	9.26-9.75	9.76-10.25	10.26-10.75	10.76-11.25	11.26-11.75	11.76-12.25			
38				1	3	8	6	17	11	11	10	10	7	9	5	1	0	1				6.555 ± .098	1.460 ± .070	20.75 ± 1.03
76				1	4	6	9	8	20	10	6	8	6	6	6	6	3	1				6.735 ± .112	1.665 ± .079	24.72 ± 1.25
79			1	2	2	9	11	18	11	11	15	10	4	1	1	1	1	1	0	0	1	6.220 ± .099	1.472 ± .070	23.66 ± 1.20
81						4	6	12	5	13	11	17	12	12	2	4	2					7.075 ± .090	1.334 ± .064	18.85 ± 0.93

tumblers. One hundred mature fronds were measured from each clone. The results of this study are shown in table 13. From table 13 it is seen that clones 38 and 76 have about the same range of variation. They also approach each other in mean size, which is 6.555±.098 mm² in clone 38 and 6.735±.112 mm² in clone 76. The standard deviations are 1.460±.070 and 1.665±.079 mm² respectively. Clone 79 has the widest range of variation and the least mean size, 6.220±.099 mm². Its standard deviation is 1.472±.070 mm². Clone 81 has the largest mean size, 7.075±0.090 mm², and the least standard deviation, 1.334±.064 mm². Clones 79 and 81 show a significant difference. The difference in the mean is .855±.134.

TABLE 14

Variation in size of fronds grown in nutrient solution and in tap water.

Clone	Period	Culture media	Frequency of class values in square millimeters (mm²)																			
			1.76-2.25	2.26-2.75	2.76-3.25	3.26-3.75	3.76-4.25	4.26-4.75	4.76-5.25	5.26-5.75	5.76-6.25	6.26-6.75	6.76-7.25	7.26-7.75	7.76-8.25	8.26-8.75	8.76-9.25	9.26-9.75	9.76-10.25	10.26-10.75	10.76-11.25
38	First	Nutrient				1	1	4	2	6	14	12	12	15	10	7	6	4	3	2	1
		Tap water	4	13	10	10	24	14	18	6	1										
	Second	Nutrient					2	7	6	11	24	20	11	11	7	1					
		Tap water		1	5	12	22	25	24	8	3										
	Third	Nutrient					6	1	11	14	21	14	8	10	6	4	4	1			
		Tap water	2	7	14	16	20	23	11	5	1	1									
41	First	Nutrient							2	3	12	14	20	11	9	12	8	4	3	0	2
		Tap water		5	14	8	17	18	14	8	11	2	1								
	Second	Nutrient				2	1	4	14	16	19	13	11	9	4	7					
		Tap water	3	7	8	20	19	23	13	4	2	1									
	Third	Nutrient			1	2	2	1	6	3	14	22	16	9	11	5	5	1	1		
		Tap water	1	4	9	14	18	15	13	5	10	3	6	1	0	1					
79	First	Nutrient					1	2	5	7	14	6	13	11	6	13	9	9	1	2	1
		Tap water	1	3	3	8	19	20	12	17	13	3	1								
	Second	Nutrient					3	7	12	10	16	11	11	6	6	5	5	3	3	2	
		Tap water	13	7	11	15	8	13	19	6	6	0	2								
	Third	Nutrient			1	1	3	3	7	7	12	13	11	12	8	11	5	3	0	2	1
		Tap water	2	9	13	20	27	19	7	1	1	1									
81	First	Nutrient			1	0	2	5	5	8	10	14	20	15	8	4	2	4	1	0	1
		Tap water	6	8	19	17	17	23	4	3	2	1									
	Second	Nutrient						2	4	4	6	12	16	19	11	8	3	6	6	2	1
		Tap water	1	5	15	13	20	19	13	9	4	1									
	Third	Nutrient				2	4	2	8	14	8	11	6	11	10	8	8	2	4	1	1
		Tap water	4	9	15	17	14	24	8	2	6	1									

Effect of culture media on clonal size variation

In the study of inheritance of acquired size in *Lemna minor,* which is reported later on in this paper, parallel cultures were grown from each clone in nutrient solution and in tap water. The materials obtained from this experiment may also be examined for the effect of different culture media on variation in size. From each of the four clones used, 38, 41, 79 and 81, three series were grown in different periods of time. One hundred mature fronds were measured from each culture. The results of these measurements are given in tables 14 and 15, the former gives the frequency distributions of the different classes found, and the latter, the different constants calculated.

TABLE 15
Constants from table 14.

Clone	Period	Culture medium	Mean	C.V.	σ	$\sigma_{nut} - \sigma_{tap}$
38	First	Nutrient	7.210 ± .102	20.87 ± 1.04	1.505 ± .072	0.524 ± .086
		Tap water	4.070 ± .066	24.10 ± 1.21	0.981 ± .047	
	Second	Nutrient	6.285 ± .067	15.88 ± 0.77	0.998 ± .047	0.271 ± .058
		Tap water	4.425 ± .049	16.43 ± 0.80	0.727 ± .035	
	Third	Nutrient	6.365 ± .085	19.81 ± 0.98	1.261 ± .060	0.367 ± .074
		Tap water	3.985 ± .060	22.43 ± 1.12	0.894 ± .043	
41	First	Nutrient	7.485 ± .086	17.01 ± 0.83	1.273 ± .061	0.198 ± .079
		Tap water	4.430 ± .073	24.27 ± 0.93	1.075 ± .051	
	Second	Nutrient	6.220 ± .078	18.55 ± 0.91	1.154 ± .054	0.245 ± .069
		Tap water	4.030 ± .061	22.55 ± 1.13	0.909 ± 0.43	
	Third	Nutrient	6.985 ± .088	18.74 ± 0.92	1.309 ± .062	0.022 ± .087
		Tap water	4.575 ± .087	28.13 ± 1.44	1.287 ± .061	
79	First	Nutrient	7.145 ± .103	21.53 ± 1.07	1.538 ± .073	0.533 ± .087
		Tap water	4.710 ± .068	21.34 ± 1.06	1.005 ± .048	
	Second	Nutrient	6.635 ± .108	24.08 ± 1.21	1.598 ± .076	0.332 ± .097
		Tap water	3.980 ± .085	31.81 ± 1.66	1.266 ± .060	
	Third	Nutrient	6.925 ± .106	22.71 ± 1.14	1.573 ± .075	0.753 ± .084
		Tap water	3.825 ± .055	21.44 ± 1.07	0.820 ± .039	
81	First	Nutrient	6.815 ± .093	20.32 ± 1.01	1.385 ± .066	0.438 ± .080
		Tap water	3.755 ± .064	25.22 ± 1.28	0.947 ± .045	
	Second	Nutrient	7.020 ± .095	20.03 ± 0.99	1.406 ± .067	0.450 ± .081
		Tap water	4.160 ± .064	22.98 ± 1.15	0.956 ± .046	
	Third	Nutrient	6.940 ± .114	24.38 ± 1.23	1.692 ± .081	0.674 ± .095
		Tap water	3.930 ± .069	25.90 ± 1.31	1.018 ± .049	

The outstanding result shown by table 15 is that, using standard deviation as the expression of variation, the plants grown in nutrient solution were always more variable in size than those grown in tap water. The differences between the standard deviations of parallel cultures are significant and are, with two exceptions, all well beyond the limits of probable error.

Inheritance of acquired size

The fact has already been pointed out that plants growing under natural conditions have demonstrated their capacity to react readily with favorable medium for growth, not only by a change in shape of the fronds but also by a considerable increase in size, amounting in some cases to more than 100 percent. Likewise it was observed that fronds previously grown in nutrient solution produced offspring which are very much smaller than themselves.

Inheritance of decreased size

An experiment to determine the inheritance of decreased size was made with clones 38 and 41 as follows: From a stock culture of each clone, the same number of buds of the same age were transferred to both tap water and nutrient media and there allowed to propagate until a sufficient harvest of mature fronds could be obtained at any one time. This constitutes the first period of the experiment. In the second period, cultures in both tap water and nutrient solutions were grown from buds from the tap water culture of the first period. At the same time a check culture in nutrient medium was grown. In the third period, tap water and nutrient cultures were similarly grown from the tap water stock of the preceding period and again a check culture was set. There are several months of interval between each two periods to give the plants time to be "acclimatized" in each new medium for growth. From each of

TABLE 16

Mean size in square millimeters of fronds from nutrient solution, grown in tap water, and of their offspring when grown again in nutrient solution.

Clone	Parent mean in nutrient solution	Offspring mean in tap water	Mean when back in nutrient solution	Check in nutrient solution
38	6.555 ± .098	4.070 ± .066	6.315 ± .066	6.775 ± .091
		4.425 ± .049	6.365 ± .085	6.450 ± .092
41	7.120 ± .073	4.430 ± .073	6.220 ± .078	6.615 ± .062
		4.030 ± .061	6.805 ± .090	6.985 ± .088

these cultures one hundred fronds were measured. Table 16 contains a summary of the results of this experiment.

From table 16 it may be concluded that decreased size acquired by nutrient fronds in their sojourn in tap water is not inherited.

Inheritance of increased size

The plan of this experiment is inversely similar to that of the inheritance of decreased size.

This experiment was carried through only two periods. As usual, one hundred mature fronds were studied from each culture. The results of the measurements are shown in table 17.

TABLE 17

Mean sizes in square millimeters of tap-water fronds grown in nutrient solution and of their offspring when grown in tap water.

Clone	Parent mean in tap water	Offspring mean in nutrient solution	Mean when back in tap water	Check in tap water
79	4.750 ± .068	7.405 ± .104	3.980 ± .085	3.825 ± .055
81	3.990 ± .066	6.815 ± .093	4.160 ± .064	3.930 ± .069

From table 17 it is seen that while starved plants grown in nutrient media increase in size by nearly 100 percent, when grown again in tap water reversion to the starved mean may be complete, showing no inheritance of the acquired increased size.

Clonal selection for size of frond

In table 13 the variations in size of clones 38, 76, 79 and 81 have already been shown. Selections for both large and small size were carried out with these four clones as an attempt to shift the means of the lines. Each selection was carried through five periods. Plus selection, or selection for large size, was made by continuously selecting individuals above the mean, and minus selection, or selection for small size, was performed by continuous selection of individuals below the mean. A check culture containing unselected individuals was also grown at the same time with the plus and minus series. The plants were grown in nutrient solution and extreme care was taken to render all controllable conditions as much alike as possible for each set of three cultures. Tables 18 to 21 contain the results of this experiment. The differences between the means of the check cultures and those of the plus and minus selections are placed in table 22 so that the effect of selection may be studied more conveniently.

Table 18

Results of continuous selection for large and small size in Clone 38.

Selection	Frequency of class values in square millimeters																				Mean	σ	C.V.
	1.76 to 2.25	2.26 to 2.75	2.76 to 3.25	3.26 to 3.75	3.76 to 4.25	4.26 to 4.75	4.76 to 5.25	5.26 to 5.75	5.76 to 6.25	6.26 to 6.75	6.76 to 7.25	7.26 to 7.75	7.76 to 8.25	8.26 to 8.75	8.76 to 9.25	9.26 to 9.75	9.76 to 10.25	10.26 to 10.75	10.76 to 11.25	11.26 to 11.75	11.76 to 12.25			
First period																							
Plus (+)						6	4	14	18	16	18	13	6	2	2	0	1					6.530±.072	1.073±.051	16.43±0.80
Check				2	4	4	3	10	22	22	11	6	5	4	3	2	1	0	0	1		6.500±.093	1.383±.066	21.28±1.06
Minus (—)					3	5	5	8	13	21	17	15	8	3	2							6.575±.075	1.107±.053	16.83±0.82
Second period																							
Plus (+)			2	2	3	5	9	11	14	13	9	10	9	7	3	1	1	1				6.485±.103	1.525±.073	23.51±1.18
Check				1	1	5	7	9	11	13	19	13	9	5	4	0	1	2				6.775±.091	1.366±.065	20.16±1.00
Minus (—)				1	4	5	10	13	15	9	9	10	13	7	1	1	1	0	1			6.535±.098	1.451±.069	22.20±1.11
Third period																							
Plus (+)						2	4	7	15	20	22	13	9	3	4	0	1					6.830±.069	1.026±.049	15.02±0.73
Check					1	1	6	13	16	22	19	10	4	3	3	1	1					6.615±.072	1.074±.051	16.23±0.79
Minus (—)				2	0	2	5	11	11	24	23	11	8	1	1	1						6.585±.069	1.022±.049	15.52±0.76
Fourth period																							
Plus (+)				1	0	3	2	3	14	18	20	15	11	5	4	3	1					7.025±.078	1.157±.055	16.47±0.81
Check					2	4	1	7	12	14	23	15	7	7	4	3	1					6.965±.082	1.218±.058	17.49±0.86
Minus (—)			1	0	0	2	4	5	15	18	28	18	3	5	0	0	0	0	1			6.755±.069	1.019±.049	15.08±0.73
Fifth period																							
Plus (+)						2	4	1	5	9	19	18	19	7	7	5	4					7.535±.081	1.202±.057	15.95±0.78
Check				1	1	4	2	6	14	12	12	15	10	7	6	4	3	2	1			7.210±.102	1.505±.072	20.87±1.04
Minus (—)			2	1	4	3	3	6	9	11	10	12	8	14	7	6	3	0	0	1		7.175±.115	1.699±.081	23.68±1.19

Table 19

Results of continuous selection for large and small size in clone 76.

Selection	Frequency of class values in square millimeters																					Mean	σ	C.V.
	1.76 to 2.25	2.26 to 2.75	2.76 to 3.25	3.26 to 3.75	3.76 to 4.25	4.26 to 4.75	4.76 to 5.25	5.26 to 5.75	5.76 to 6.25	6.26 to 6.75	6.76 to 7.25	7.26 to 7.75	7.76 to 8.25	8.26 to 8.75	8.76 to 9.25	9.26 to 9.75	9.76 to 10.25	10.26 to 10.75	10.76 to 11.25	11.26 to 11.75	11.76 to 12.25			
First period																								
Plus (+)						2	3	5	5	9	13	11	13	16	9	6	5	2	1			7.740±.095	1.413±.067	18.25±0.90
Check							4	5	5	12	13	15	16	9	8	6	5	1	1			7.645±.090	1.331±.063	17.41±0.85
Minus (—)				1	1	3	4	7	10	15	11	11	11	7	6	6	4	2	1			7.275±.106	1.568±.075	21.55±1.07
Second period																								
Plus (+)								2	3	7	9	8	19	15	10	8	7	3	3	3	3	8.485±.100	1.478±.070	17.42±0.85
Check						2	2	3	5	8	9	12	14	15	7	9	4	5	4	1		8.070±.104	1.541±.073	19.09±0.94
Minus (—)						1	1	3	8	9	10	11	12	20	10	6	4	2	2	1		7.930±.093	1.385±.066	17.46±0.86
Third period																								
Plus (+)						1	4	4	5	12	18	14	11	11	7	5	3	2	2	1		7.625±.096	1.425±.068	18.69±0.92
Check						1	2	3	10	17	19	25	7	6	4	3	2	1				7.245±.074	1.101±.053	15.20±0.74
Minus (—)					1	1	3	7	8	17	13	17	7	9	7	5	4	1				7.330±.091	1.355±.065	18.48±0.91
Fourth period																								
Plus (+)				1	2	3	7	11	16	16	12	9	9	5	5	2	1	1				6.715±.093	1.377±.066	20.51±1.02
Check					4	2	8	13	18	14	8	9	4	4	8	6	2					6.745±.102	1.509±.072	22.37±1.12
Minus (—)				1	4	6	13	11	14	11	11	9	8	4	1	6	1					6.465±.100	1.483±.071	22.94±1.15
Fifth period																								
Plus (+)				1	1	1	1	7	10	11	13	13	14	8	7	5	3	2	1	1	1	7.520±.105	1.552±.074	20.64±1.02
Check					1	1	4	5	5	7	18	19	12	13	9	4	1	1				7.465±.084	1.250±.060	16.70±0.82
Minus (—)						1	3	8	10	10	17	16	9	8	6	5	3	2	1	1		7.430±.097	1.431±.068	19.26±0.97

Table 20

Results of continuous selection for large and small size in clone 79.

Selection	Frequency of class values in square millimeters																				Mean	σ	C.V.
	1.76 to 2.25	2.26 to 2.75	2.76 to 3.25	3.26 to 3.75	3.76 to 4.25	4.26 to 4.75	4.76 to 5.25	5.26 to 5.75	5.76 to 6.25	6.26 to 6.75	6.76 to 7.25	7.26 to 7.75	7.76 to 8.25	8.26 to 8.75	8.76 to 9.25	9.26 to 9.75	9.76 to 10.25	10.26 to 10.75	10.76 to 11.25	11.26 to 11.75	11.76 to 12.25			
First period																							
Plus (+)				1	2	1	5	15	11	12	16	11	6	7	6	2	3	2				6.940±.099	1.472±.070	21.21±1.05
Check					1	3	7	5	11	12	20	14	13	5	5	2	2					7.000±.085	1.262±.060	18.03±0.89
Minus (—)				1	1	5	6	16	11	8	12	12	11	8	4	2	1	1	1			6.830±.100	1.484±.071	21.73±1.08
Second period																							
Plus (+)					1	3	4	8	11	17	18	8	12	5	4	3	3	1	1	0	1	7.110±.099	1.470±.070	20.67±1.03
Check					1	1	4	5	15	13	20	12	11	9	4	2	1	1	1			7.120±.085	1.263±.060	17.74±0.87
Minus (—)						3	6	11	11	15	19	9	9	7	6	1	1	1	0	1		6.955±.091	1.344±.064	19.32±0.95
Third period																							
Plus (+)			1	0	1	2	5	12	10	8	12	13	8	7	11	4	1	2	1	1	1	7.270±.113	1.674±.080	23.01±1.15
Check					1	6	9	5	12	12	16	12	13	9	3	0	2					6.835±.088	1.312±.063	19.19±0.95
Minus (—)				1	2	4	8	7	16	12	10	13	7	7	6	3	1	1	1	0	1	6.915±.107	1.588±.076	22.96±1.15
Fourth period																							
Plus (+)					2	1	5	6	16	11	11	11	7	5	9	3	4	6	1	1	1	7.435±.118	1.745±.083	23.47±1.18
Check					1	1	6	11	13	13	13	12	9	4	6	4	2	3	2			7.145±.103	1.533±.073	21.45±1.07
Minus (—)				4	2	4	1	8	15	8	13	11	7	10	10	4	1	1	1			7.055±.110	1.629±.078	23.09±1.16
Fifth period																							
Plus (+)				1	1	2	3	6	9	6	12	7	4	7	7	12	6	13	3	1		8.040±.130	1.920±.092	23.88±1.20
Check					1	2	5	7	14	6	13	11	6	13	9	9	1	2	1			7.405±.104	1.538±.073	20.77±1.03
Minus (—)				1	1	2	3	10	15	14	12	10	6	6	5	5	4	4	1	1		7.230±.112	1.662±.079	22.99±1.15

Table 21

Results of continuous selection for large and small size in clone 81.

Selection	Frequency of class values in square millimeters																					Mean	σ	C.V.
	1.76 to 2.25	2.26 to 2.75	2.76 to 3.25	3.26 to 3.75	3.76 to 4.25	4.26 to 4.75	4.76 to 5.25	5.26 to 5.75	5.76 to 6.25	6.26 to 6.75	6.76 to 7.25	7.26 to 7.75	7.76 to 8.25	8.26 to 8.75	8.76 to 9.25	9.26 to 9.75	9.76 to 10.25	10.26 to 10.75	10.76 to 11.25	11.26 to 11.75	11.76 to 12.25			
First period																								
Plus (+)					4	1	3	7	10	13	17	11	8	8	9	6	1	1	1			7.220±.100	1.477±.070	20.46±1.02
Check			1	0	2	5	5	8	10	14	20	15	8	4	2	4	1	0	1			6.815±.093	1.385±.066	20.32±1.01
Minus (—)					2	1	7	8	11	8	25	13	8	8	5	3	0	1				6.985±.086	1.279±.061	18.31±0.90
Second period																								
Plus (+)			1	2	4	6	3	9	13	12	18	16	9	3	2	1	1					6.550±.092	1.358±.065	20.73±1.03
Check				2	2	5	5	12	16	15	19	9	5	6	4							6.500±.083	1.232±.059	18.95±0.93
Minus (—)			1	1	2	8	13	9	16	10	14	14	4	3	3	2						6.330±.091	1.342±.064	21.20±1.05
Third period																								
Plus (+)					2	8	6	6	15	11	24	12	10	1	3	0	1	1				6.630±.085	1.256±.060	18.94±0.93
Check			1	0	3	3	7	13	25	15	15	6	4	2	1	2	2	1				6.405±.088	1.298±.062	20.26±1.00
Minus (—)					2	3	6	9	14	17	16	19	7	5	2							6.670±.074	1.091±.052	16.36±0.80
Fourth period																								
Plus (+)					1	2	5	6	11	15	13	11	11	5	4	3	7	4	2			7.375±.109	1.612±.077	21.86±1.09
Check						1	3	4	13	15	15	15	10	7	7	5	2	2	1			7.370±.090	1.334±.064	18.10±0.89
Minus (—)					1	1	3	9	11	11	19	13	8	7	6	5	4	1	1			7.280±.095	1.415±.067	19.44±0.96
Fifth period																								
Plus (+)					1	3	4	8	18	13	11	11	11	8	5	4	2	1				7.035±.093	1.378±.066	19.59±0.97
Check				1	1	1	8	9	13	15	15	14	5	5	4	4	2	1	1	1		6.970±.101	1.495±.071	21.45±1.07
Minus (—)				1	2	6	8	12	18	22	14	6	5	2	1	2	1					6.340±.081	1.194±.057	18.83±0.93

TABLE 22

Differences in square millimeters between the means of the checks and those of the selections.

Period	Difference between means of check and plus selection	Difference between means of check and minus selection
	Clone 38	
First	0.030 ± .117	—0.075 ± .119
Second	—0.290 ± .137	0.240 ± .134
Third	0.215 ± .100	0.030 ± .100
Fourth	0.060 ± .113	0.210 ± .107
Fifth	0.325 ± .130	0.035 ± .154
	Clone 76	
First	0.095 ± .131	0.370 ± .139
Second	0.415 ± .144	0.140 ± .139
Third	0.380 ± .121	—0.085 ± .117
Fourth	—0.030 ± .138	0.280 ± .143
Fifth	0.055 ± .135	0.035 ± .128
	Clone 79	
First	—0.060 ± .130	0.170 ± .131
Second	—0.010 ± .130	0.165 ± .124
Third	0.435 ± .143	—0.080 ± .138
Fourth	0.290 ± .157	0.090 ± .151
Fifth	0.635 ± .166	0.175 ± .153
	Clone 81	
First	0.405 ± .136	—0.170 ± .127
Second	0.050 ± .124	0.170 ± .123
Third	0.225 ± .123	—0.265 ± .115
Fourth	0.005 ± .156	0.090 ± .147
Fifth	0.065 ± .137	0.630 ± .130

If the data in table 22 are examined, and if a significant difference between a selection mean and the mean of a corresponding check is assumed to be at least three times the probable error, it will be found in clone 38 that while the plus means were generally greater and the minus means generally smaller than the means of the checks, no single significant difference was obtained. In clone 76 there seems to be only one important difference, that for the plus selection in the third period. It is hard to account for this seeming effect of selection since in the last two periods of the selection, the difference was not maintained. Moreover, it will be observed that in the same (third) period, the minus series had a greater mean than the check, indicating that some factor, probably cultural, had affected the growth of the plants in the check culture, thus

rendering the opposite differences both unusual. In clone 79 there was entirely no effect of selection in the minus series. In the plus experiments, significant differences were obtained in the third and fifth periods, which may be considered as showing that selection was slightly effective. In clone 81 selection was of no avail. The single important difference obtained in the last period of the minus selection was probably due to the fact that some sort of fungous disease attacked the plants of the minus culture and the effect of the disease on size was not entirely overcome in sampling.

On the whole, it may be concluded that the results of this experiment, to shift the mean size of a clone, showed a very doubtful effect of selection without excluding the possibility that such an effect may be possible.

DISCUSSION AND CONCLUSIONS

Lemna minor is a convenient material for clonal study. It can be grown in artificial media in the laboratory, propagates fairly rapidly and, owing to its small size, it lends itself readily to extensive but well controlled observation and measurements without requiring much laboratory space. In many respects, it is comparable to Paramecium. One advantage it has over the latter is that one can always be sure with it that he is harvesting or sampling for measurement fully matured individuals which have therefore attained their mature size. In Paramecium, there is no way of determining that all the individuals being studied are absolutely fully grown. This fact subjects Paramecium measurement to a grave error, for in comparing the mean size of a group with that of another, the mean size is influenced by the number of immature animals, and it may readily be seen that if one of the groups propagates faster than the other, the former will have at any one time more young individuals than the latter.

While this plant can be grown in tap water alone and in tap water containing soil, the most satisfactory culture medium found, which can be controlled, is a modified PFEFFER's solution. The gradual dwindling of the plants when grown in tap water, especially when no frequent change of this is made, may be due to real lack of mineral food or to the absence of some organic growth-promoting substance which is now called an auximone. BOTTOMLEY (1917) in a recent paper found the presence of this substance essential to the normal and long-time growth of *Lemna minor* in DETMER's standard mineral solution. By placing

water extract of bacterized peat in such a solution, he was able to get continuous, luxuriant growth. Contrary to BOTTOMLEY'S conclusion that *Lemna minor* cannot maintain normal growth in a mineral solution without auximones, it has been grown in this experiment in a mineral solution without the addition of any other substance. BOTTOMLEY'S conclusion is unfair since he did not show that he used the other known standard mineral solutions, any one of which, as the present experiment has proved, may suit the normal growth of the plant.

The characters used as variants in this work are size and shape of frond, and the speed of budding. The length of root is a very unsatisfactory if not a useless character for this purpose on account of its characteristic and probably hereditary twisting habit.

While different strains in shape and size of frond and speed of propagation have been found to exist in a population, the number of these strains is not as large as might at first be imagined. The area of the natural habitat from which the material is collected is undoubtedly an important factor in the obtaining of a larger number of elementary strains, if such larger number exists. The smaller this area is, the more chance there is of finding the population in a high state of freedom from mechanical mixture since, owing to the rapid propagation of the plant, and under the influence of natural selection, a clone may be easily established at any one favorable spot.

Results of clonal selection to shift the mean in speed of propagation or to change the dominant shape of a clone have confirmed the pure line theory. The results of size selection, on the other hand, have not been in entire accord with JOHANNSEN'S idea.

Unusual variations in shape have been observed, but they were not inherited, showing that they were merely somatic or physiological variations.

Lemna minor has been found to respond readily to different culture media. By growing it in an artificial or mineral solution, its natural size has been increased more than 100 percent and the predominating shape of a clone changed, as well as the speed of asexual reproduction hastened. Under such favorable conditions for growth, there was found greater variability in shape and size than under less favorable conditions. Moreover, acquired size, as a result of a change in growing medium, appeared to be non-heritable.

SUMMARY

1. There have been found different races in a population of *Lemna minor* as regards size and shape of frond and speed of propagation.

2. No single case of mutation has been observed in this experiment which covers a period of one and one-half years and which involved several thousands of individuals.

3. In a clone, there was found greater variability among plants grown in a mineral solution than among those grown in tap water.

4. Decreased or increased size acquired by plants through a change of cultural environment during less than a year's time was not inherited.

5. The results obtained in clonal selection either in shape of frond or in speed of propagation are in accord with the pure line theory. Selection to shift the mean size has shown slight effect in one out of ten cases.

The writer is deeply indebted to Professor C. H. MYERS not only for suggesting the subject of this investigation but also for liberal help in securing some of the apparatus and material used, as well as for valuable suggestions and criticisms throughout the experiment and the preparation for this paper. Thanks are also due to Professor H. H. LOVE for aid in some of the biometrical work and to Professor O. F. CURTIS for calling the author's attention to BOTTOMLEY'S work.

LITERATURE CITED

BLODGETT, F. H., 1915 Morphology of the Lemna frond. Bot. Gaz. **60**: 383-390.

BOTTOMLEY, W. B., 1917 Some effects of organic growth-promoting substances (auximones) on the growth of *Lemna minor* in mineral culture solutions. Proc. Roy. Soc. B **89**: 481-507.

GRAY, A., 1908 New manual of botany. A handbook of the flowering plants and ferns of the Central and Northern United States and adjacent Canada. 7th Ed. New York: American Book Co.

GUPPY, H. B., 1894 On the habits of *Lemna minor, L. gibba* and *L. polyrrhiza*. Jour. Linn. Soc. London **30**: 323-330.